

Functional and Evolutionary Implications of the Distribution of Phosphagens in Primitive-Type Spermatozoa

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Abstract. External fertilization is considered to be the primitive condition in metazoans. The spermatozoa of such organisms typically display a common primitive-type morphology that is present in a range of phyla. These spermatozoa are extremely polarized cells in that the site of ATP synthesis (mitochondria in midpiece) is located at large diffusion distances from the ATP sink (dynein ATPases in the flagellum). Spermatozoa of polychaetes, sipunculids, echiuroids, echinoderms, and tunicates contain the phosphagen creatine phosphate or express the corresponding phosphagen kinase creatine kinase (or both), even when other phosphagens/phosphagen kinases are present in somatic tissues and eggs. The selective expression of the creatine kinase system in these spermatozoa may be related to potential advantages in the cellular transport of energy. To evaluate this possibility, we compared the efficacy of the major phosphagen systems for cellular transport of energy. We used a facilitated diffusion model for spatial ATP buffering, taking into account relative differences in diffusivity and thermodynamic poise. At low ratios of [total phosphagen pool]/[total adenine nucleotide pool] (C_{G+P}/C_{Ad} ratio), creatine phosphate carried a higher fraction of total high-energy phosphate (J) than the other phosphagens. However, J values for all phosphagens were greater than 0.9, and these differences disappeared as the C_{G+P}/C_{Ad} ratio was increased. Thus, the functional benefit of using CP, rather than other phosphagens, in energy transport is quite limited. The creatine kinase system became associated with primitive-type spermatozoa early in metazoan evolution.

This association is not necessarily related to inherent advantages of this phosphagen system for buffering of ATP, but may be linked to historical events in the evolution of the cell phenotype.

Introduction

The energy “content” of ATP is not fixed but rather is related to the extent of displacement of the hydrolysis reaction ($ATP \rightarrow ADP + \text{inorganic phosphate}$) from thermodynamic equilibrium (Nicholls and Ferguson, 1992), as the effective free energy of ATP hydrolysis (ATP chemical potential, ΔG_{ATP}) is a function of the physiological [ATP]/[ADP] ratio. Under normal conditions, cellular energy metabolism maintains the hydrolysis reaction far displaced from equilibrium, which is reflected by high prevailing [ATP]/[ADP] ratios and correspondingly high values for ΔG_{ATP} (Nicholls and Ferguson, 1992; Kammermeier, 1993). Physiological conditions that lead to increases in cellular ADP concentrations lead to disproportionately larger decreases in the [ATP]/[ADP] ratio. The net effect is substantial reductions in ΔG_{ATP} that dramatically impact the functioning of critical ATP-requiring processes in cells (Kammermeier *et al.*, 1982; Kammermeier, 1987, 1993).

Phosphagens are phosphorylated guanidine compounds that are involved in buffering cellular ATP levels. These compounds are typically found in cells with high and variable rates of energy turnover. A diverse array of phosphagens is found in the animal kingdom, including arginine phosphate (AP), creatine phosphate (CP), glycoyaminate phosphate (GP), taurocyamine phosphate (TP), hypotaurocyamine phosphate (HTP), and lombricine phosphate (LP). Corresponding phosphagen (guanidino)

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kinases—arginine kinase [AK], creatine kinase [CK], glycoamine kinase [GK], taurocyamine kinase [TK], hypotaurocyamine kinase [HTK] and lombricine kinase [LK]—catalyze the reversible transfer of phosphate from phosphagen to ADP, yielding ATP (phosphagen + ADP \leftrightarrow ATP + guanidine acceptor).

The traditional view of phosphagen systems is that they function as temporal ATP buffers (Meyer *et al.*, 1984). Typically, phosphagen concentrations are higher than adenine nucleotide concentrations (Beis and Newsholme, 1975; Meyer *et al.*, 1984). During periods of intense energy turnover, ΔG_{ATP} is kept high by the transphosphorylation of ADP to ATP at the expense of the phosphagen. This role of the phosphagen system has been likened to that of the discharge of a capacitor (Meyer, 1988; Kammermeier, 1993). Ellington (1989) showed that the apparent equilibrium constants ($K' = ([ATP] * [guanidine]) * ([phosphagen] * [ADP])^{-1}$) for the phosphagen kinase reactions span a range of values from 13.2 (AK) to 100 (CK); others are intermediate. This means that the CK reaction can buffer ATP over higher ranges of ΔG_{ATP} than other phosphagen systems (Ellington, 1989). However, CP might be viewed as being disadvantageous in certain invertebrate systems in which lower pH values and reduced set-point ΔG_{ATP} values may prevail, thereby inducing rapid dissipation of the CP pool (Wyss *et al.*, 1992).

A second hypothesized function of phosphagens is a potential role (particularly CP) in the transport of high-energy phosphate from "source" (typically mitochondria) to "sink" (ATPases). One perspective on the transport role, formulated as the CP "shuttle" (Bessman and Geiger, 1981) and later CP "circuit" (Wallimann *et al.*, 1992), hypothesizes that there are separate pools of adenine nucleotides at source and sink and that the CP/CK system provides the energetic link between the two sites. The net effect is to allow rapid transport of high-energy phosphate while maintaining appropriate ΔG_{ATP} values at source and sink (Mainwood and Rakuson, 1982; Jacobus, 1985; Kammermeier, 1987). The above arguments are based on diffusive constraints (concentration gradients; membrane permeability) of the adenine nucleotides vs. CP/creatine in relation to energy turnover rates. The basic premise for this view is that intracellular diffusion of ATP and particularly ADP is limiting. That is, the conditions that optimize diffusive movement of the adenine nucleotides (high concentration gradients) would produce ΔG_{ATP} values at the site of ATP utilization (sink) that would be incompatible with functioning of the ATPase (Jacobus, 1985; Kammermeier, 1987). Furthermore, additional diffusive constraints for ADP, such as binding to intracellular surfaces and reduced permeability for entry into the mitochondrion, have been identified (Saks *et al.*, 1993, 1996).

Phosphagens are distributed along distinct phylogenetic

lines (for reviews see Watts, 1968, 1971, and 1975). The CP/CK system is found in the vertebrates, lower chordates, and lower and higher invertebrate groups. The AP/AK system is widely distributed throughout the invertebrates and is present in lower chordates but absent in the vertebrates. The remaining phosphagen systems are primarily found in vermiform groups best typified by polychaetes, which as a group have all phosphagen systems (Watts, 1968, 1971, 1975). Of great interest is the observation that in such groups as polychaetes, echinoderms, and tunicates, regardless of which phosphagen system is present in somatic cells and eggs, the CP/CK system is always present in the primitive-type spermatozoa of these animals (Watts, 1968, 1971, 1975; Tombes and Shapiro, 1989). It has long been recognized that the diffusion of ATP (and ADP) might limit motility in cells that use flagella and cilia (Raff and Blum, 1968; Nevo and Rickenspoel, 1970; Lin, 1972). Thus, energy transport by phosphagens should be especially important in more highly polarized cells such as primitive-type spermatozoa, which have mitochondria in the midpiece and dynein ATPases distributed along the flagellum (Tombes and Shapiro, 1989). Here we evaluate differences in the energy transport properties of the various phosphagen systems in an effort to explain the selective expression of the CP/CK system in these primitive-type spermatozoa.

Materials and Methods

Rationale for approach

The functioning of the phosphagen systems in energy transport should be dependent on a number of parameters—thermodynamic properties of the phosphagen kinase reaction (Meyer *et al.*, 1984; Ellington, 1989) and diffusive constraints such as relative diffusivities and concentration differences. Thermodynamic differences have been documented (Ellington, 1989). The various phosphagens span a range of relative molecular masses (M_r). According to the Stokes-Einstein equation, diffusivity (diffusion coefficient, D) is given by the following expression:

$$D = k*T/(6*\pi*n*r)$$

where

k = Boltzman's constant,

T = absolute temperature,

n = viscosity,

r = molecular radius

Molecular volume should be roughly proportional to M_r . If one assumes a spherical molecule, then D values for each phosphagen should be proportional to the cubed root of the M_r . In the present study we empirically determined

the relative diffusivities of five major phosphagens and ATP. We then used these data to model the functional impact of differences in D , K' , and phosphagen concentrations on energy transport, using the steady-state, facilitated diffusion model of Meyer *et al.* (1984), in which energy transport by phosphagens is viewed as spatial ATP buffering.

Phosphagens

Five major phosphagens were used in this study (see Fig. 1 for chemical structures and Mr values). CP and AP are available commercially from Sigma Chemical Co. (St. Louis, MO). The remaining phosphagens were enzymatically synthesized using the appropriate guanidine acceptor and phosphagen kinase. We followed the protocol of Poat *et al.* (1980) with an ATP-regenerating system consisting of phosphoenolpyruvate and pyruvate kinase. CK catalyzes the phosphorylation of glycoeyamine, albeit at a much lower rate than creatine. Thus, GP was synthesized using glycoeyamine (Sigma) and muscle CK (Boehringer Mannheim, Indianapolis, IN). LK catalyzes the phosphorylation of both lombricine and taurocyamine (Suzuki *et al.*, 1997). LK was purified from the body wall of the earthworm *Eisenia foetida* according to the protocol of Suzuki *et al.* (1997). Lombricine was isolated as a natural product from the body wall of the earthworm *Lumbricus terrestris* according to the protocol of Ellington (1989). Purity was verified using thin-layer chromatography, NMR spectroscopy, and enzymatic assay. Taurocyamine was synthesized as previously described (Ellington, 1989). Both LP and TP were enzymatically

synthesized using LK as described above. All other biochemical reagents were from Sigma or Boehringer Mannheim.

Measurement of diffusion coefficients using pulsed gradient NMR spectroscopy

Diffusion coefficients for phosphagens and ATP were determined using a Bruker DMX-WB600 NMR spectrometer equipped with XYZ gradients. Phosphagens and ATP were dissolved in a model solution consisting of 50 mM HEPES buffer, 200 mM KCl, 100 mM glycine, and 25 mM $MgCl_2$ (pH of solution adjusted to 7.4). Samples were placed in 10-mm NMR tubes and mounted in a microimaging probe. Phosphorus NMR spectra were acquired at 242 MHz. Temperature was maintained at 20°C. Diffusion coefficients were determined with pulsed gradient spin echo NMR (PGSE) using a bipolar gradient sequence with a longitudinal eddy current delay period (BPP-LED) (Wu *et al.*, 1995). In this protocol, spin echo experiments were conducted with increasing applied gradients that resulted in attenuation of the ^{31}P -NMR signal. The slope of this attenuation curve is directly proportional to the mobility of the molecule being observed. Due to this dependence on mobility, it was possible to calculate the D values under these circumstances. Since AP, GP, TP, and LP have nearly identical chemical shifts, these phosphagens were investigated in independent experiments.

Mathematical treatments

Numerical simulations were conducted using Mathcad Plus version 6 (Mathsoft, Cambridge, MA) on a Pow-

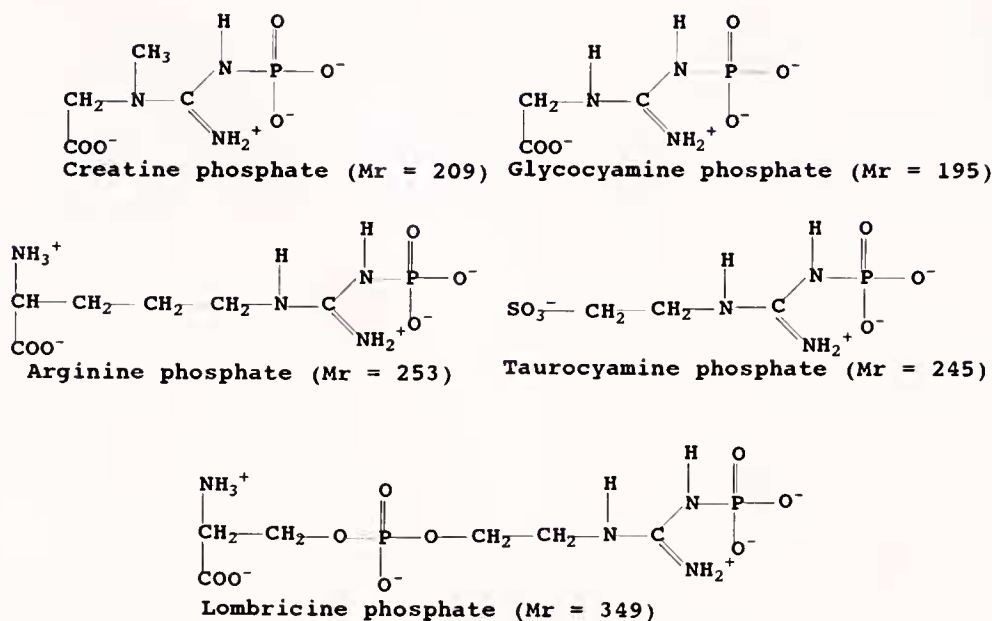


Figure 1. Structures of five of the major phosphagens.

erMac 7600 microcomputer and SlideWrite Plus version 4 (Advanced Graphics Software, Carlsbad, CA) on a Pentium 133 MHz microcomputer. Equations and boundary conditions are described in the text.

Results and Discussion

Diffusion coefficient

PGSE NMR determinations of diffusion coefficients for phosphagens and ATP yielded very consistent results within replicates for each treatment (Table I; Fig. 2). As expected according to the Stokes-Einstein equation, the D values were indirectly proportional to Mr. That is, there was a linear relationship between the diffusion coefficient and the reciprocal of the cube root of the Mr (Fig. 2). In the context of cellular energy transport, the more physiologically relevant parameter is the ratio $D_{\text{phosphagen}}/D_{\text{ATP}}$, which reflects relative mobilities of the "high energy" phosphates. The ratios range from 1.222 for the largest phosphagen, LP, to 1.597 for the smallest, GP (Table I). The $D_{\text{PC}}/D_{\text{ATP}}$ ratio observed in the present study is highly comparable to values obtained in model solutions by other groups (Yoshizaki *et al.*, 1987; Moonen *et al.*, 1990; Hubley *et al.*, 1995).

Simulation of spatial ATP buffering

Meyer *et al.* (1984) pointed out that under conditions of submaximal rates of aerobic energy turnover, the role of phosphagens can be viewed as a form of facilitated diffusion in which the phosphagen and its corresponding guanidine base, in effect, substitute for ATP and ADP, respectively. If one assumes that the phosphagen kinase reaction is globally near equilibrium throughout the cell,

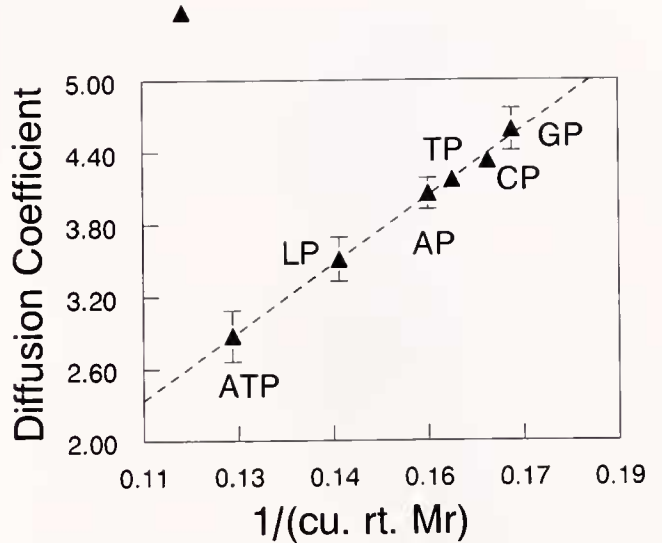


Figure 2. Relationship between diffusion coefficient ($\times 10^{-6}$ cm²/s) and the cubed root of the relative molecular mass. Each value represents a mean ± 1 SD (*n* size is reported in Table I).

then the importance of phosphagens in energy transport can be described by the following relation (Meyer *et al.*, 1984):

$$J_{\text{phos.}}/J_{\text{ATP}} = (D_{\text{phosphagen}}/D_{\text{ATP}}) * (C_{\text{G+P}}/C_{\text{Ad}}) * K' * [(R + 1)/(R + K')]^2$$

where

- $J_{\text{phos.}}/J_{\text{ATP}}$ = ratio of phosphate carried by the phosphagen over phosphate carried by ATP
- $C_{\text{G+P}}/C_{\text{Ad}}$ = ratio of concentration of total phosphagen pool over total adenine nucleotide pool
- K' = apparent equilibrium constant of phosphagen kinase
- $R = [\text{ATP}]/[\text{ADP}]$ ratio

In this context, it is assumed that the diffusion coefficients for phosphagen vs. guanidine base and ATP vs. ADP are not substantially different (Meyer *et al.*, 1984). Implicit in our use of *in vitro* D values and $D_{\text{phosphagen}}/D_{\text{ATP}}$ ratios is the assumption that these ratios do not change appreciably *in vivo*. This assumption appears to be valid for $D_{\text{PC}}/D_{\text{ATP}}$ in vertebrate skeletal muscle (Hubley *et al.*, 1995). The Meyer *et al.* (1984) model was originally developed to look at high-energy phosphate diffusion in skeletal muscle fibers. Although the geometry of spermatozoa is somewhat different, we consider the application of the model appropriate in the context of comparing relative capacities for energy transport amongst the phosphagens.

For the purposes of establishing boundary conditions, we chose a physiologically reasonable value of 300 for the [ATP]/[ADP] ratio. Boundary limits for $D_{\text{phosphagen}}/D_{\text{ATP}}$ ratios and K' were 1.1 to 1.6 and 10 to 100, respectively, which bracket the range of values in Table I. For

Table I

Properties of phosphagens and corresponding phosphagen kinase reactions; data for diffusion coefficients (D) correspond to means ± 1 SD

	D (cm ² /s) $\times 10^{-6}$	$D_{\text{phosphagen}}/D_{\text{ATP}}$	Apparent equilibrium constant ¹
ATP	2.872 \pm 0.212 (<i>n</i> = 23)	—	—
Lombrocin phosphate	3.509 \pm 0.184 (<i>n</i> = 12)	1.222	32.3
Arginine phosphate	4.054 \pm 0.129 (<i>n</i> = 5)	1.412	13.2
Taurocyamine phosphate	4.169 \pm 0.063 (<i>n</i> = 6)	1.452	27.3
Creatine phosphate	4.329 \pm 0.089 (<i>n</i> = 5)	1.508	100
Glyocyamine phosphate	4.586 \pm 0.177 (<i>n</i> = 6)	1.597	29.0

¹ From Ellington (1989).

values of the C_{G+P}/C_{Ad} ratio we chose a range of values of 1 to 8, which brackets the observed pool ratios for a range of contractile/motile cells (Beis and Newsholme, 1975; Tombes *et al.*, 1985; Hamm and Yue, 1987; Schneider *et al.*, 1989). Numerical solutions to the above equation were calculated and expressed as the fraction of total high-energy phosphate flux carried by the phosphagen (J).

Figure 3 depicts the relationship between J, $D_{\text{phosphagen}}/D_{\text{ATP}}$ ratio, and K' at a $C_{G+P}/C_{Ad} = 1$ and a $[ATP]/[ADP]$ ratio = 300. It is apparent that there is a steep dependence of J on K' , especially in the lower K' range. The dependence of J on relative diffusivity is much less pronounced (Fig. 3). When one plots the individual 3-dimensional coordinates for each phosphagen type on the response surface, the positions of each are well dispersed (Fig. 3). However, CP's position on the surface shows that it carries the highest fraction of phosphate while AP carries the lowest. The other three phosphagens show intermediate J values (Fig. 3). Note that under the conditions used in the Figure 3 simulation, all of the phosphagens are responsible for transporting over 90% of high-energy phosphate. The impact of incrementally increasing C_{G+P}/C_{Ad} ratios on J is shown in Figure 4. The dependence of J on the $D_{\text{phosphagen}}/D_{\text{ATP}}$ ratio and K' becomes increasingly compressed so that these latter two parameters have minimal impact at higher C_{G+P}/C_{Ad} ratios.

The above analyses were conducted using the limiting condition of the ATP/ADP ratio being equal to 300. Although within physiological limits, this ratio reflects an upper extreme of energy state condition that may exist in

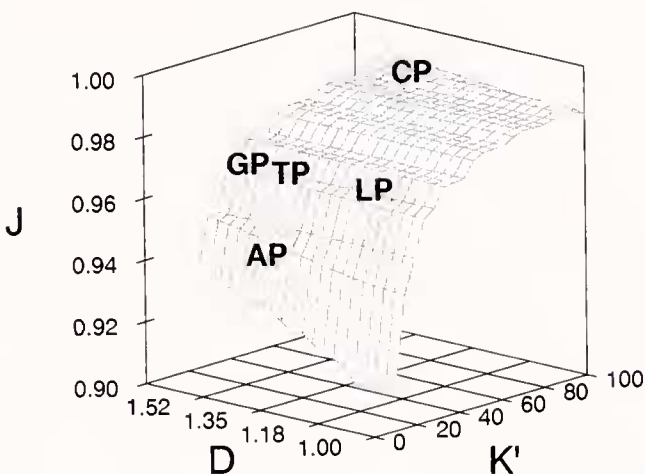


Figure 3. Impact of $D_{\text{phosphagen}}/D_{\text{ATP}}$ ratio (denoted D in figure) and apparent equilibrium constant (K') on the fraction of total high-energy phosphate (J) carried by phosphagens according to the facilitated diffusion model of Meyer *et al.* (1984). ATP/ADP and C_{G+P}/C_{Ad} ratios were set at 300 and 1, respectively. Specific J values for the various phosphagens (CP, GP, TP, LP, and AP) were calculated based on their respective D and K' values. The positions of these phosphagens are indicated on the response surface.

cells. Thus, we modeled J at a range of ATP/ADP ratios (10–300). The results (Table II) show that differences in the transport capabilities of the phosphagens are minimal except at the lowest ATP/ADP ratio (10). In this extreme case, the CK system is the least effective in transport, as was first noted by Meyer *et al.* (1984).

Before assessing the physiological implications of these results, it is useful to comment on the validity of the above simulation. In addition to the facilitated diffusion approach, high-energy phosphate transport *in vivo* has also been investigated extensively using reaction-diffusion models (Raff and Blum, 1968; Nevo and Rikenspoel, 1970; Lin, 1972; Tombes *et al.*, 1985; Hubley *et al.*, 1997); these models require the use of *in vivo* D values that are typically somewhat lower than corresponding *in vitro* D values (Moonen *et al.*, 1990; Hubley *et al.*, 1995). Furthermore, intracellular barriers dramatically decrease *in vivo* D values in a time-dependent manner (Kinsey *et al.*, 1998), which must be taken into account in reaction-diffusion analyses of energy transport. However, in our analyses we used $D_{\text{phosphagen}}/D_{\text{ATP}}$ ratios that should be minimally affected by such restrictions to diffusion and, consequently, would not compromise our overall conclusions.

Furthermore, the facilitated diffusion model is applicable only to situations in which aerobic energy turnover is near or below maximal and the global cellular high-energy phosphate concentrations do not change appreciably (or at least reach a new steady state). These circumstances should prevail in primitive-type spermatozoa of externally fertilizing marine animals that must swim for a considerable time in the water column. Recently, van Dorsten *et al.* (1997) showed that activation of motility in spermatozoa from the sea urchin *Psammechinus miliaris* is accompanied by dramatic increases in both respiration and the forward flux of CK (CP → ATP). In the same study, CP and ATP remained essentially constant for at least 2 h of motility, an observation consistent with steady-state energy transport by the CP/CK system according to the facilitated diffusion model.

Phosphagen kinase equilibrium state and compartmentation are other issues to be considered. The Meyer *et al.* (1984) approach assumes global equilibrium of the phosphagen kinase reaction throughout the cell. A number of workers have questioned the validity of this assumption (Saks and Aliev, 1996; Aliev and Saks, 1997). Recent evidence suggests that a significant fraction of the pool of creatine/creatine phosphate is not in equilibrium with CK in skeletal muscle fibers (Hochachka and Mossey, 1998). Furthermore, the whole issue of diffusion as a paradigm for intracellular transport has recently been questioned (Agutter *et al.*, 1995). In some sense, these issues are experimentally intractable in the sperm system and cannot be addressed in this study. However, on the basis of cell morphology and rates of energy turnover, it

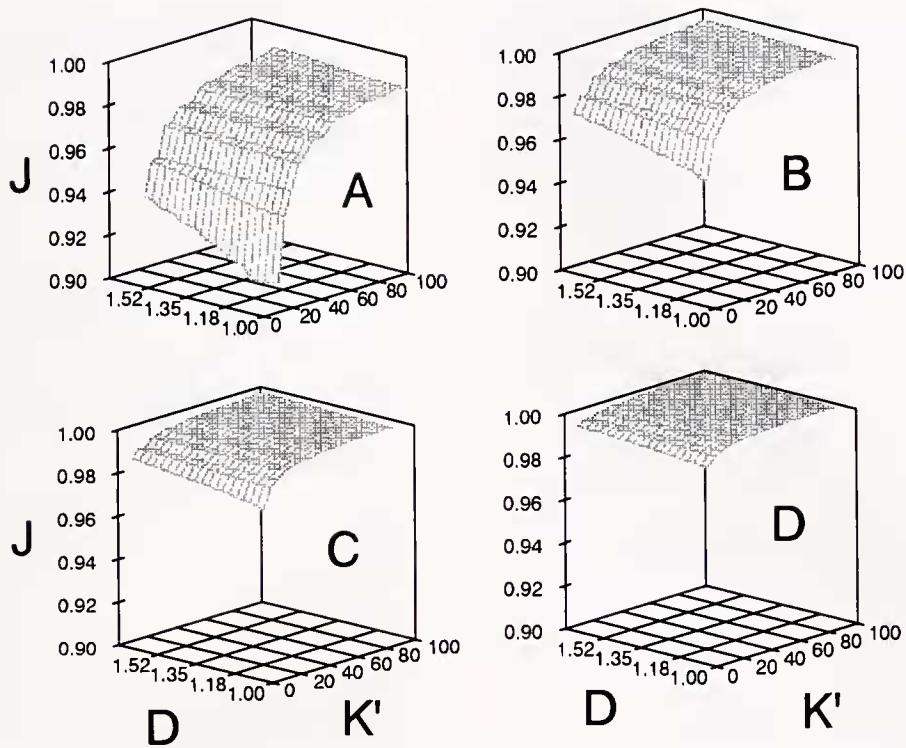


Figure 4. Impact of changes in the C_{G+P}/C_{Ad} ratio on fraction high-energy phosphate transport by phosphagens: (A) $C_{G+P}/C_{Ad} = 1$; (B) $C_{G+P}/C_{Ad} = 2$; (C) $C_{G+P}/C_{Ad} = 4$; and (D) $C_{G+P}/C_{Ad} = 8$. ATP/ADP ratio and Y and X axes are the same as in Fig. 3.

seems clear that there are certain diffusion restrictions on energy transport in primitive-type spermatozoa. Thus, in the present study we use the facilitated diffusion model to look only for broadscale and relative differences between the phosphagen systems—differences that would change the overall diffusive component of energy transport.

Diffusion of phosphagens in physiological context

The above results show that differences in thermodynamic potential and intrinsic diffusivity impact the rela-

tive capacity of phosphagens in facilitated energy transport under typical conditions of ΔG_{ATP} . At lower C_{G+P}/C_{Ad} ratios, CP, because of its higher diffusivity and high K' , carries a greater fraction of high-energy phosphate (J) than other phosphagens. However, even at low C_{G+P}/C_{Ad} ratios J values for all phosphagens fall well above 0.9. Furthermore, these differences become negligible as the C_{G+P}/C_{Ad} ratio is increased (Fig. 4). Meyer *et al.* (1984) pointed out that the spatial ATP buffering role of phosphagens should be “most important in large cells with nonuniform mitochondrial distribution in which diffusion distances are greatest.” Our analyses above show that spatial ATP buffering is moderately dependent on phosphagen type but strongly influenced by the C_{G+P}/C_{Ad} ratio. As indicated previously, primitive-type spermatozoa are highly polarized, with mitochondria separated by large diffusion distances from the dynein ATPases in the flagellum; yet these cells are capable of sustained aerobic energy turnover during swimming. Elegant work by Tombes and Shapiro (1985) and Tombes *et al.* (1985) showed that sea urchin spermatozoa contain creatine kinase (CK) and that sustained swimming is highly dependent on CK activity, presumably due to its role in energy transport.

The CP/CK system is known to be associated with primitive-type spermatozoa. Even though other phosphagen systems may be present in somatic tissues and eggs,

Table II

Impact of changes in the setpoint ATP/ADP ratio on the transport capacities of phosphagen kinase reactions

ATP/ADP ratio	LK	AK	TK	CK	GK
10	0.870	0.913	0.896	0.791	0.902
25	0.953	0.956	0.961	0.942	0.964
50	0.974	0.968	0.977	0.977	0.979
100	0.982	0.974	0.984	0.990	0.986
200	0.987	0.976	0.987	0.994	0.989
300	0.988	0.977	0.989	0.995	0.990

For the purpose of these calculations, the [total phosphagen pool]/[total adenine nucleotide pool] (C_{G+P}/C_{Ad} ratio) was set at 2.5. Data are listed as fraction of the total high-energy phosphate carried by the phosphagen (J, as in Figures 3 and 4).

the spermatozoa of all echinoderms, tunicates, and polychaetes consistently have the CP/CK system only (Watts, 1971; Tombs and Shapiro, 1989; Kamp *et al.*, 1995), with CK activities ranging from 7.1 to 30 $\mu\text{moles} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ in echinoderms (Ratto *et al.*, 1989). The sipunculid *Sipunculus nudus* has high activities of AK in its somatic tissues (van Thoai *et al.*, 1966) yet the spermatozoa contain CK only (G. Kamp, pers. comm.). Recently, we have shown that sperm of another sipunculid, *Phascolopsis gouldii*, contain significant CK activity ($16.6 \mu\text{moles} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$) while muscle contains HTK activity (W. R. Ellington, unpub. obs.). This phenomenon is also characteristic of sperm of the echiuroid *Urechis caupo*, in which CK activity approaches $10 \mu\text{moles} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ (W. R. Ellington, unpub. obs.) even though the phosphagen system present in muscle is LP/LK (Robin, 1964; van Thoai *et al.*, 1972).

The above pattern, as seen in major protostome (polychaetes, echiuroids, and sipunculids) and deuterostome (echinoderms, tunicates) groups, indicates that when an organism has the potential to express more than one phosphagen type, if CP/CK is one of the possibilities, it is the type that will, without exception, be expressed in spermatozoa. Thus, the selective expression of the CP/CK system in cells where spatial ATP buffering is critical (using logic of Meyer *et al.*, 1984) could be related to adaptive differences in functional properties amongst the phosphagen systems.

The above adaptationist interpretation of CP/CK system distribution in primitive-type spermatozoa is completely undermined by our analyses, above, which show that the differences in transport properties of the various phosphagen systems are small and that these differences collapse as the C_{G+P}/C_{Ad} ratio is increased. Marine bivalve molluscs have highly motile primitive-type spermatozoa that contain high AK activities (Tombs and Shapiro, 1989). The motility of these spermatozoa does not appear to be compromised by the lack of the CK/CP system. In 1971, Watts pointed out a distinct advantage for sperm to contain CP instead of AP: creatine is a dead-end compound, whereas arginine is in the mainstream of amino acid metabolism and is clearly important in sperm due to histone biosynthesis. This argument is credible for the preference of CP over AP in many spermatozoa but fails to explain the exclusion of phosphagens derived from glycocyamine, taurocyamine, hypotaurocyamine, and lombricine, all of which are dead-end compounds.

Why is CK selectively expressed in many spermatozoa?

Spermatozoa demonstrate a diverse array of morphologies and metabolic capacities that correlate well with mode of fertilization and—for species with external fertilization—the nature of the microhabitat (Kamp *et al.*, 1996; Kaldis *et al.*, 1997). For instance, mammalian sper-

matozoa have very low or no CK activity but generally have a high capacity for fermentative pathways (Kamp *et al.*, 1996; Kaldis *et al.*, 1997), which decreases the effective polarity ATP source and sink. Carp sperm have low CK activity but show motility for only a few minutes after release (Kamp *et al.*, 1996). In contrast, phosphagen kinase activities (especially CK) are much higher in the sperm of externally fertilizing marine invertebrates (Tombs and Shapiro, 1989; Kamp *et al.*, 1996). This dependency on phosphagen kinase is best exemplified by echinoderm sperm, which rely exclusively on mitochondrial fat oxidation (Kaldis *et al.*, 1997) and must sustain long periods of motility in the water column.

Regardless of which model is utilized, the constraints on the diffusion of adenine nucleotides make phosphagen systems critical in the motility of many primitive-type spermatozoa. However, the present simulations suggest that the various phosphagen systems differ only slightly in their capacities for energy transport, as modeled in the context of facilitated diffusion, and that these differences collapse when the C_{G+P}/C_{Ad} ratio is increased (see Fig. 4). Thus, we are hard-pressed to ascribe a functional advantage in terms of energy transport to the possession of the CP/CK system rather than another phosphagen system.

Echinoderm spermatozoa contain an octameric mitochondrial CK isoform (Tombs and Shapiro, 1985; Wyss *et al.*, 1995) that is very similar to the vertebrate mitochondrial CK present in somatic tissues (Wyss *et al.*, 1992, 1995). A unique "contiguous" trimeric CK is found restricted to the flagellum (Tombs and Shapiro, 1985; Wothe *et al.*, 1990). We recently discovered that the spermatozoa of the polychaete *Chaetopterus variopedatus* have octameric mitochondrial and "contiguous" trimeric flagellar CKs which have strikingly similar physical properties to echinoderm sperm CKs (Ellington *et al.*, 1998). Although definitive conclusions about the evolutionary relationships amongst these mitochondrial and flagellar CK's must await completion of our determinations of cDNA sequences for the polychaete forms, it seems likely that both the mitochondrial and flagellar CK isoforms evolved prior to the divergence of protostomes and deuterostomes, at least 670 million years ago (Doolittle *et al.*, 1996).

A number of workers have recently pointed out the uncertainties of attempting to view physiological characters in extant animals as adaptations to current environmental situations (Huey, 1987; Harvey and Pagel, 1991). Historical issues have to be considered. External fertilization is thought to be the primitive metazoan condition (Baccetti and Afzelius, 1976) and primitive-type spermatozoa are very similar in structure regardless of group. It is tempting to speculate that the expression of the CK system may reflect some ancient linkage between the cell

phenotype and the expression of the suite of cell-specific genes, including the two CK isoforms.

Acknowledgments

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